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TITLE: Use of GDNF-Releasing Nanofiber Nerve Guide Conduits for the Repair of
Conus Medullaris/Cauda Equina Injury in the Non-Human Primate

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14. ABSTRACT Cauda equina and conus medullaris forms of spinal cord injury result in paralysis, sensory impairment, and autonomic dysfunction. This study investigates the effects of neural repair in nonhuman primates using a GDNF-releasing nerve guidance channel. These studies aim to repair avulsed lumbosacral ventral roots using a bridging strategy. For comparison, the studies will also include the use of a guidance channel without GDNF release and a peripheral nerve graft to bridge the tissue gap. A comprehensive set of electrodiagnostic, imaging, behavioral and anatomical studies will provide detailed information about the outcome of these interventions. During the second year of this project, our studies have continued to make significant progress. We developed an algorithm for presurgical testing of nonhuman primates, including locomotor treadmill studies, pain behavioral assessments, urodynamic recordings, MRI studies, and anal sphincter EMG studies. We also demonstrated the feasibility of lumbosacral ventral root avulsion procedures. We have demonstrated the feasibility of using peripheral nerve grafts and GDNF-releasing nerve guide conduits to bridge tissue gaps between the spinal cord and avulsed ventral roots. All 20 surgeries have been completed and collections of comprehensive functional and imaging data are in progress.					
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Table of Contents

	<u>Page</u>
Introduction.....	3
Body.....	3-5
Key Research Accomplishments.....	5
Reportable Outcomes.....	5-6
Conclusion.....	6
References.....	6-7
Appendices.....	7-8

Progress Report Summary of USAMRMC Protocol Number SC090273P2, Award Number W81XWH-10-1-0907 entitled, "Neural Repair in a Primate Model of Cauda Equina Injury"

INTRODUCTION

Cauda equina and conus medullaris forms of spinal cord injury result in paralysis, sensory impairment, and autonomic dysfunction (Hoang and Havton, 2006; Havton and Carlstedt, 2009). The present study investigates the effects of neural repair in the non-human primate using a GDNF-releasing nerve guidance channel. The studies aim to repair avulsed lumbosacral ventral roots using a bridging strategy. For comparison, the studies will also include the use of a guidance channel without GDNF release and a peripheral nerve graft to bridge the tissue gap. A comprehensive set of electrodiagnostic, imaging, behavioral and anatomical studies will provide detailed information about the outcome of the intervention. We are hopeful that this translational research study may guide planning of future clinical studies on neural repair after cauda equina/conus medullaris injuries. The present report will summarize the major accomplishments within the second year of this 3-year project.

BODY

Our studies are going well and significant progress is being made. At the beginning of the reporting period, Dr. Havton moved from UCLA to UC Irvine, where he had accepted a position as Professor and Vice Chair for Research in the Department of Anesthesiology & Perioperative Care. Dr. Havton also joined, as a member, the Reeve-Irvine Research Center for Spinal Cord Injury Research. In this collaborative partnership project, Dr. Havton interacts regularly with the participating PIs (Dr. Kari Christe at the California Primate Research Center at UC Davis and Dr. Ahmet Höke at Johns Hopkins University).

At the onset of the project, research subjects in the form of adult female rhesus monkeys were selected for pre-surgical testing and enrollment in the studies. The selection and screening process is extensive, as behavioral components are very important for the success of the studies. Unfortunately, not all subjects that are initially assessed were able to pass the behavioral criteria needed for successful participation in our study. However, during the first year of the project, we developed an algorithm to ensure selection of suitable subjects for the study. Here, it is important to acknowledge that the pre-surgical selection, screening, and testing of our subjects is thorough and time consuming. However, we have been successful in developing a method for optimal subject selection, trained staff to perform the behavioral screening and evaluation, as well as successfully implemented our procedures for animal enrollment and testing. This algorithm for animal selection was also followed during year 2.

As stated above, prior to the start of surgical procedures, each animal undergoes extensive screening and training. Behavioral records and profiles are reviewed to select subjects that are likely to cooperate with all aspects of the training. Next, each animal is introduced to transfers, from cage to a carrying cage using a chute. This is followed by the introduction of the treadmill environment, which is enclosed by a plexi-glass cage. Animals are trained to walk on the treadmill belt at various speeds, and

desired behavior is encouraged using various food rewards. Training requires multiple sessions and extensive variation exists between subjects with regards to how many sessions are needed for a subject to be a reliable treadmill walker. Extra time for training is sometimes allocated to accommodate for individual variation. However, when a subject requires additional sessions for treadmill training, it may delay the start of the other behavioral training sessions, e.g. chair training for pain screening.

Successful treadmill training is a requirement for subsequent chair training, which is needed in order to perform sensory testing using von Frey hairs and an Electro-von-Frey device to obtain baseline sensory thresholds pre-operatively. In addition, the chair training is needed for the application of paint markers over the hip, knee, ankle, distal metatarsal bone, and distal portion of the fifth toe. The paint markers are essential for filming of treadmill walking to obtain digital recordings for subsequent kinematics analysis.

Electromyography (EMG) recordings of the external anal sphincter are obtained pre-operatively as baseline records. The external anal sphincter muscle is chosen as it is directly affected by the ventral root injury and will undergo partial denervation as a result of the unilateral lumbosacral ventral root avulsion injury in our experimental model. The development of external anal sphincter recordings in the non-human primate was developed by us during this project and reflects innovation and a new outcome measure for studies in monkeys. The data from control subjects are presently in the late stages of preparation for a research manuscript aimed at submission this fall (2012).

We have also developed a method for urodynamic recordings in the non-human primate. The continued development and refinement of the urodynamic procedures have primarily taken place during year 2 of this project. This is quite an achievement, as no previously established methods are present in the literature. We have developed a method for performing cystometrogram recordings, urethra pressure recordings, and external urethral sphincter EMG recordings in anesthetized subjects. In addition, we have developed a method for obtaining external abdominal wall EMG recordings during the procedures and are therefore able to monitor and screen for potential visceral pain development. These studies are novel and promising and will represent a very useful additional outcome measure. Similar to our EMG studies of the external anal sphincter, the urodynamic studies are reportable as scientific findings. We are presently analyzing the data from the pre-surgical series with the goal of preparing a research manuscript on the normal physiology of the lower urinary tract in nonhuman primates. Data collected during the post-operative phase are being collected as well and will be prepared for subsequent manuscripts on injury and repair studies for this translational research model.

Magnetic resonance imaging (MRI) is performed of the lower spine to visualize the lumbosacral spinal cord and associated nerve roots as well bilateral hindlimbs to visualize muscles groups both above and below the level of the knee. The MRI recordings of the spinal cord and nerve roots pre-and post-operatively will allow us to monitor nerve root degeneration and axonal regeneration associated with cauda equina injury and repair. We anticipate that the MRI studies may be able to identify successful muscle reinnervation prior to functional improvement taking place.

All 20 subjects adult female rhesus monkeys were selected, screened, and successfully completed full pre-surgical testing, including MRI, treadmill training, chair testing, and pain screening using a von Frey testing approach.

All 20 surgical procedures have been completed with post-operative times currently ranging from 2 weeks to 14 months. The majority of subjects were operated on during year 2. These subjects represent both experimental and control groups, including subjects undergoing surgery with ventral root injury and repair using our GDNF-releasing nerve guidance conduits or peripheral nerve grafts. Our comprehensive outcome measures, including pain testing, locomotor treadmill studies, urodynamic studies, EMG recordings, and MRI studies are currently ongoing in this large group of subjects. The project endpoint is 18 months post-operatively at which point tissue samples will be collected.

KEY RESEARCH ACCOMPLISHMENTS

We developed an algorithm for selection of animals based on behavioral and treadmill locomotor criteria.

We developed a method for obtaining interpretable quantitative EMG recordings from the external anal sphincter pre- and post-operatively. In addition, we developed comprehensive urodynamic methods, which allow for screening for visceral pain in addition to obtaining functional micturition data. These novel data will also be prepared for a research manuscript within the next reporting cycle.

Collection of comprehensive pre-surgical data, including treadmill locomotor studies with an automated digital recording system, imaging of the lumbosacral spinal cord and lower extremity muscles using MRI, collection of urodynamic recordings, EMG recordings of the external anal sphincter, and sensory threshold testing using manual von Frey hair and Electro-von-Frey approaches

We demonstrated that surgical use of peripheral nerve grafts and of GDNF-releasing nerve guidance conduits are feasible as bridges between the spinal cord and their avulsed ventral roots in nonhuman primates. These surgical procedures were generally tolerated well (one animal developed an unexplained liver hemorrhage though it was not thought to be related to this surgery).

REPORTABLE OUTCOMES

Most of the reportable data outcomes will be at the end of the post-operative study period, as longitudinal data are being collected, including locomotor behavior, EMG recordings, pain behavioral monitoring, and MRI studies as well as morphological outcome measures after the collection of nerve root and spinal cord tissues. However, some of our collected pre-surgical data are novel and reflect innovation and new knowledge. For instance, our baseline anal sphincter EMG and comprehensive urodynamic recordings are examples of new and original findings.

We are currently preparing a manuscript describing evoked activation of external anal sphincter EMG activity in rhesus macaques and provide support and rationale for these studies to serve as quantifiable outcome measures in primate spinal cord injury and repair models.

We are also preparing a manuscript that demonstrates feasibility of performing comprehensive urodynamic studies in non-human primates, and that such functional outcome measures of micturition reflexes are suitable for studies of spinal cord injury and repair in non-human primates.

We have presented an abstract and poster at the 2012 Military Health System Research Symposium that was held August 13-16, 2012, in Fort Lauderdale, FL. The title of our presentation was:

"Use of GDNF-Releasing Nanofiber Nerve Guide Conduits for Repair of Conus Medullaris/Cauda Equina Injury in the Non-Human Primate" by L.A. Havton, J.H. Nieto, M. Ohlsson, H.H. Chang, H.Q. Mao, A Höke, and K.L. Christe

All participating Principal Investigators, i.e. Dr. L.A. Havton, Dr. K.L. Christe, and Dr. A. Höke were also present and participated in the MHSRS/ATACCC meeting in Fort Lauderdale.

We have also presented an abstract/poster at the American Urological Association Annual Meeting in Atlanta, Georgia, May 19-23, 2012. The title of our presentation was:

"Evoked Electromyographic Activity in the External Anal Sphincter Muscle of Non-Human Primates Differs from Corresponding Patterns in Humans" by U. Lee, H.H. Chang, K.L. Christe, and L.A. Havton

CONCLUSION

Our studies have continued to make significant progress. We developed an algorithm for pre-surgical testing of nonhuman primates, including locomotor treadmill studies, pain behavioral assessments, urodynamic recordings, MRI studies, and anal sphincter EMG studies. We also demonstrated the feasibility of a lumbosacral ventral root avulsion procedure with animals recovering well after the surgical spine and spinal cord procedure. We have also demonstrated the feasibility of using peripheral nerve grafts and GDNF-releasing nerve guide conduits to bridge tissue gaps between the spinal cord and avulsed ventral roots. All 20 surgeries for this study have been completed and collections of comprehensive functional and imaging data are in progress for the planned study time of 18 months (for each subject). We will continue our ongoing data collection as planned for the third year of our studies.

In addition to ongoing data collection, collected data are already being prepared for initial publications on important novel outcome measures, including external anal sphincter EMG recordings and comprehensive urodynamic studies in this translational research model.

REFERENCES

Havton LA, Carlstedt T. Repair and rehabilitation of plexus and root avulsions in animal models and patients. *Curr Opin Neurol* 2009, 22: 570-574.

Hoang TX, Havton LA. Novel repair strategies to restore bladder function following cauda equina/conus medullaris injuries. *Prog Brain Res* 2006, 152: 195-204.

SUPPORTIVE DATA



